Research Paper

Synthesis, Spectral Characterization and Antimicrobial Studies of New Hybrid Heterocyclic Compounds Bearing 1H-benzimidazol-2-yl Thiomethyl Motif

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To understand the biological importance of heterocyclic cores, novel 1H-benzimidazol-2-yl thiomethyl incorporated hybrid compounds, 2-(benzimidazol-2-ylthiomethyl)-5-aryl-1,3,4-oxadizoles and 1-(2-(1H-benzo[d]imidazol-2-ylthio)acetyl)pyridazine/phthalazinediones were designed using molecular hybridization technique, synthesized and characterized. The compounds were screened for *in vitro* antimicrobial activity using the serial dilution technique and were found to exhibit weak antitubercular activity, excellent to moderate antibacterial and better antifungal activities against some tested organisms in comparison to the standard drugs. Thus, some of the title compounds demonstrated antimicrobial activity.

Key words: 1,3,4-oxadiazole, phthalazine, pyridazine, molecular hybridization, serial dilution, antitubercular drugs

Many infections are caused by pathogenic organisms like bacteria, fungi, and viruses. Even though antibiotics prescribed for the treatment of such diseases are lifesaving, some of these have harmful side effects leading to allergy, anaphylactic reaction, superinfections, destruction of normal bacterial flora of the body and selective toxicities like anaemia and renal damage. Moreover, the development of multiple drug resistance by causative organisms with time makes the existing antibiotics ineffective[1]. Therefore, development of effective, safe and economic antimicrobial agents is the need of the hour.

Heterocycles, over the years, have contributed to mankind to understand life processes and improving the quality of life. Exploration of new heterocycles that can interact with multiple biological targets remains an intriguing scientific endeavour. In particular, sulphur and nitrogen containing heterocycles remained to be the mainstay of continued interest by researchers[2]. Being structural surrogates of nucleotides, benzimidazole derivatives exhibited the potential to interact with proteins, enzymes and receptors. Substituted benzimidazoles were found to possess various biological activities such as antibacterial[3], antiinflammatory[4], antifungal[5], antitubercular[6], anticancer[7], anticonvulsant and antidiabetic[8]. A number of 2-thio-1H-benzimidazole derivatives have been demonstrated to exhibit potent antiparasitic[9,10] activities. Diversely 2,5-disubstituted-1,3,4-oxadiazoles have not only been reported to possess antibacterial[11,12], antifungal[13] and antibrucella[14] actions but also served as building blocks for heterocyclic molecules. Likewise, compounds consisting of pyridazine and phthalazine cores too were reported to exhibit antiplatelet[15], antitumor[16] and antifungal[17] activities.

Moreover, it is noticed in many instances that incorporation of a biologically active moiety into another active molecule could result in altered biological activity due to changes brought in the physicochemical properties by the incorporated moiety[18-20]. Molecular hybridization is an emerging strategy in drug design and development that involves combination of distinct pharmacophore moieties of different active molecules to produce a new hybrid compound with improved affinity and efficacy, when compared to

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the parent molecules. It is useful in the design of new optimized ligands and prototypes with new molecular architectures. This strategy can result in compounds with modified selectivity profile, different and/or dual modes of action and reduced side effects[21-23]. Enthused by these facts, the present study has been taken up to synthesize an effective and possibly safer hybrid molecular framework consisting two active scaffolds, 1,3,4-oxadiazole/pyridazine/phthalazine and 1H-benzimidazol-2-ylthiomethyl motif, based on molecular hybridization strategy followed by structural characterization and antimicrobial evaluation.

MATERIALS AND METHODS

The 1H-2-mercaptobenzimidazole and other chemicals purchased from S. D. Fine Chem., Mumbai, India were of laboratory reagent grade and used as received. Brain heart infusion (BHI) and Middlebrook 7H9 broths were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Alamar Blue was purchased from Sigma-Aldrich, St. Louis, USA. Ciprofloxacin and fluconazole were obtained as gift samples from Lanova Chem., Mumbai, India while isoniazid (INH) was from KP Pharmaceuticals, Bidar, India.

Progress of the reaction and purity of the products were ascertained by thin-layer chromatography (TLC) using silica gel G as stationary phase and various solvent combinations as mobile phase; the spots were visualized by iodine vapours. All yields refer to crude products before purification. Melting points were determined in an open capillary melting point apparatus, Veego VMP-DS, Mumbai, India and are uncorrected. The infrared (IR) spectra were taken on Fourier transform infrared spectroscopy (FTIR) 8400S (Shimadzu Corporation, Tokyo, Japan) using the KBr pellet technique. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained in DMSO-*d6* on Bruker AvanceII 400 MHz spectrometer (Illinois, USA). Chemical shifts were measured on δ scale in parts per million (ppm) downfield to tetramethylsilane. Peak multiplicities were indicated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet) and m (multiplet). Carbon-13 nuclear magnetic resonance (^{13}CNMR) spectra were taken at 50 MHz. The mass spectra were recorded on AB Sciex API 2000 LC/MS (Framingham, USA) by ESI technique. CLogP values were computed from ChemDraw software[24]. *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212**,** *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Aspergillus niger* ATCC 1015, *Candida albicans* ATCC 10231 and *Mycobacterium tuberculosis H37Rv* ATCC 25618 were used for antimicrobial studies.

Preparation of 2-(1H-benzo[d]imidazol-2-ylthio) acetic acid 2:

A mixture of 2-mercapto benzimidazole (15 g, 0.1 mol), sodium hydroxide (4 g, 0.1 mol) and alcohol (95%, 150 ml) was refluxed for 1 h and then cooled to room temperature. Chloroacetic acid (9.14 g, 0.1 mol) was added to reaction mixture and refluxed again for 4-5 h, cooled and poured into ice cold water, acidified with dilute acetic acid and kept in refrigerator overnight. The solid separated as glistening white crystals was filtered, washed with cold water and recrystallized by ethanol. Yield: 93%; melting point: 203-205°.

Preparation of 2-(1H-benzo[d]imidazol-2-ylthio) acetyl chloride 3:

To compound 2 (20 g, 0.1 mol) thionyl chloride (21.6 ml, 0.12 mol) was added slowly during 1 h under reflux. The contents were mixed well during this period. The excess thionyl chloride was distilled off and the product was cooled and dried. Yield: 71%; melting point: 300°.

Preparation of 2-(1H-benzo[d]imidazol-2-ylthio) aceto hydrazide 4:

A mixture of compound 3 (19 g, 0.88 mol) and hydrazine hydrate (66 g, 1.32 mol) was refluxed for 15 min. Ethanol (95%) was added to get clear solution and refluxed further for 3 h. The excess of ethanol was distilled off and cooled. The obtained solid was recrystallized from ethanol (95%). Yield: 63%; melting point: 159-162°.

Preparation of 1,3,4-oxadiazole derivatives 5a-5j:

A mixture of compound 4 (2.67 g, 0.012 mol), aromatic acid (0.01 mol) and phosphorus oxy chloride (2 ml, 0.01 mol) was refluxed for 5 h and cooled. The reaction mixture was poured into ice water mixture, neutralized with dilute $NaHCO_3$. The solid obtained was filtered, washed with cold water, dried and recrystallized from ethanol (95%).

2-((1H-benzo[d]imidazol-2-ylthio)methyl)-5-phenyl-1,3,4-oxadiazole) 5a:

Prepared from benzoic acid (1.22 g, 0.01 mol) as brown needles; yield: 32%; IR, cm^{-1}**:** 3173.64 (NH str), 3085.45 (ArCH str), 1675.16 (C=N str), 1382.18 (NH bend), 744.57 (CS str); ^{1}H NMR, δ 11.52 (s, 1H, 1'H), 8.26 (s, 2H, 2', 6'H), 8.11-8.09 (m, 5H, 5', 8',

3", 4", 5"H), 7.69-7.67 (d, 2H, *J*=8.2 Hz, 6', 7'H), 3.98 (s, 2H, SCH_2); ^{13}C NMR, δ 163.8 (C5), 158.2 (C2), 148.3 (C2'), 138.2 (C4', 9'), 129.6 (C3", 5"), 128.1 (C2", 4", 6"), 122.6 (C1'', 6', 7'), 114.8 (C5', 8'), 35.2 (CS); MS, *m/z*: 308.09 (100%, M^+), 309.07 (18.2%, M^++1), 310.06 (3.9%, M^++2), $(M)^+$ found = 308.09, $C_{16}H_{12}N_4OS$ requires 308.07.

3-(5-((1H-benzo[d]imidazol-2-ylthio)methyl)-1,3,4-oxadiazol-2-yl)aniline 5b:

Prepared from *m*-amino benzoic acid (1.37 g, 0.01 mol) as creamy coloured powder; yield: 46%; IR, cm^{-1}: 3178.80 (NH str), 3085.45 (ArCH str), 1627.15 (C=N str), 1382.18 (NH bend), 1267.05 (COC str), 744.57 (CS str); ^1H NMR, δ 10.52 (s, 1H, 1'H), 7.63 (d, 2H, *J*=8.6 Hz, 5', 8'H), 7.38-7.42 (d, 2H, *J*=5.98 Hz, 2", 3"H), 7.21 (d, *J*=8.6 Hz, 2H, 6', 7'H), 6.86 (s, 1H, 2"H), 6.52 (m, 1H, 4"H), 3.8 (s, 2H, NH_2), 4.32 (s, 2H, SCH_2); ^{13}C NMR, δ 163.2 (C5) 156.3 (C2), 150.0 (C2'), 148.6 (C3''), 140.3 (C4', 9'), 130.5 (C5''), 129.3 (C1"), 124.2 (C6',7'), 118.7 (C4"), 117.3(C6"), 114.8 (C5', 8'), 113.5 (C2''), 34.7 (CS); MS, *m/z*: 323.09 (100%, M^+), 324.09 (16.8%, M^++1), 325.08 (5.2%, M^++2), $(M)^+$ found = 323.09, $C_{16}H_{13}N_5OS$ requires 323.08.

4-(5-((1H-benzo[d]imidazol-2-ylthio)methyl)-1,3,4-oxadiazol-2-yl)aniline 5c:

Prepared from *p*-amino benzoic acid (1.37 g, 0.01 mol) as brown crystals; yield: 41%; IR, cm^{-1}: 3166.24 (NH str), 3085.45 (ArCH str), 1598.89 (C=N str), 1374.38 (NH bend), 1262.42 (COC str), 742.04 (CS str); ^1H NMR, δ 12.52 (s. 1H, 1'H), 7.58-7.53 (m, 4H, 5', 8', 2", 6"H), 7.25 (d, 2H, *J*=8.4 Hz, 6', 7'H), 6.82 (d, *J*=6.42 Hz, 2H, 3", 5"H), 4.22 (s, 2H, SCH_2), 3.68 (s, 2H, NH_2); ^{13}C NMR, δ 164.2 (C5), 159.6 (C2), 148.3 (C2'), 144.9 (C4"), 140.1(C4', 9'), 128.2 (C2'', 6''), 123.4 (C6', 7'), 115.8 (C1''), 114.7 (C5', 8', 3", 5"), 35.3 (CS); MS, *m/z*: 323.07 (100%, M^+), 324.09 (17.3%, M^++1), 325.08 (5.2%, M^++2), $(M)^+$ found = 323.07, $C_{16}H_{13}N_5OS$ requires 323.08.

2-((1H-benzo[d]imidazol-2-ylthio)methyl)-5-(2-chlorophenyl)-1,3,4-oxadiazole 5d:

Prepared from *o*-chloro benzoic acid (1.56 g, 0.01 mol) as white powder; yield: 29%; IR, cm^{-1}: 3156.68 (NH str), 3085.45 (ArCH str), 1629.81 (C=N str), 1344.88 (C=N bend), 740.60 (CS str), 637.01 (CCl str); ^1H NMR, δ 11.23 (s, 1H, 1'H), 7.78 (d, *J*=6.18 Hz, 1H, 6"H), 7.62-7.83 (m, 3H, 5', 8', 2''H), 7.35-7.20 (m, 4H, 6', 7', 4'', 5''H), 4.16 (s, 2H, SCH_2); ^{13}C NMR, δ 163.6 (C5), 157.8 (C2), 150.1 (C2'), 137.5 (C4', 9'), 138.2 (C1"), 132.2 (C2"), 130.7 (C4"), 129.8 (C3"), 128.5 (C6"), 126.9 (C5"), 123.8 (C6', 7'), 115.3 (C5', 8'), 35.5 (CS); MS, *m/z*: 342.04 (100.0%, M^+), 343.02 (16.9%, M^++1), 344.03 (35.8%, M^++2), 345.04 (M^++3, 7.5%), 346.03 (M^++4, 1.9%) $(M)^+$ found = 342.04, $C_{16}H_{11}ClN_4OS$ requires 342.03.

2-((1H-benzo[d]imidazol-2-ylthio)methyl)-5-(3-nitrophenyl)-1,3,4-oxadiazole 5e:

Prepared from *m*-nitro benzoic acid (1.67 g, 0.01 mol) as brown powder; yield: 63%; IR, cm^{-1}: 3136.63 (NH str), 3085.45 (ArCH str), 1623.05 (C=N str), 1345.29 (NH bend), 1264.32 (COC str), 896.33 (CS str), 740.60 (N=O sym str); ^1H NMR, δ 12.41 (s, 1H, 1'H), 8.43 (s, 1H, 2"H), 8.40 (t, 1H, 6"H), 8.31 (d, *J*=9.26 Hz, 1H, 4"H), 7.82 (t, 1H, 5"H), 7.57 (m, 2H, 5', 8'H), 7.18 (d, 2H, *J*=8.2 Hz, 6', 7'H), 4.40 (s, 2H, SCH_2); ^{13}C NMR, δ 165.2 (C5), 159.3 (C2), 150.0 (C2"), 148.3 (C3"), 138.9 (C4',9'), 132.8 (C6"), 131.5 (C5"), 124.0 (C1"), 123.1 (C2",4", 6', 7'), 115.9 (C5', 8'), 33.8 (CS); MS, *m/z*: 353.08 (100.0%, M^+), 354.07 (23.1%, M^++1), 355.06 (3.9%, M^++2), $(M)^+$ found = 353.08, $C_{16}H_{11}N_5O_3S$ requires 353.06.

2-((1H-benzo[d]imidazol-2-ylthio)methyl)-5-(3-bromophenyl)-1,3,4-oxadiazole 5f:

Prepared from *m*-bromo benzoic acid (2.01 g, 0.01 mol) as brown powder; yield: 34%; IR, cm^{-1}: 3180.63 (NH str), 3085.45 (ArCH str), 1623.87 (C=N str), 1345.29 (NH bend), 1265.88 (COC str), 738.14 (CS str), 516.53 (CBr str); ^1H NMR, δ 11.32 (s, 1H, 1'H), 7.98 (d, 1H, *J*=6.78 Hz, 6''H), 7.62-7.41 (m, 5H, 5', 8', 2'', 4'', 5''H), 7.18 (d, 2H, *J*=8.4 Hz, 6', 7'H), 4.17 (s, 2H, SCH_2); ^{13}C NMR, δ 164.4 (C5), 158.7 (C2), 150.2 (C2'), 139.0 (C4', 9'), 133.7 (C2"), 131.6 (C5"), 128.5 (C1", 4"), 126.2 (C5"), 122.1 (C6', 7'), 119.6 (C3"), 115.8 (C5', 8'), 34.9 (CS); MS, *m/z*: 387.95 (100.0%, M^+), 388.97 (16.3%, M^++1), 389.98 (5.2%, M^++2), 385.96 (96.3%), 386.99 (18.3%), $(M)^+$ found = 387.95, $C_{16}H_{11}BrN_4OS$ requires 385.98.

4-(5-((1H-benzo[d]imidazol-2-ylthio)methyl)-1,3,4-oxadiazol-2-yl)phenol 5g:

Prepared from *p*-hydroxy benzoic acid (1.38 g, 0.01 mol) as pale yellow powder; yield: 25%; IR, cm^{-1}: 3760.38 (OH str), 3353.65 (NH str), 3085.45 (ArCH str), 1633.22 (C=N stretch), 1386.04 (NH bend), 1252.97 (COC str), 739.95 (CS str); ^1H NMR, δ 10.53 (s, 1H, 1'H), 8.27 (s, 1H, OH), 7.99 (d, 2H, *J*=7.27 Hz, 2", 6"H),

7.73 (d, 2H, *J*=8.6 Hz, 5', 8'H), 7.42 (d, *J*=8.6 Hz, 2H, 6', 7'H), 7.02 (d, 2H, *J*=7.27 Hz, 3'', 5''H), 3.55 (s, 2H, SCH_2); ^{13}C NMR, δ 164.5 (C5), 162.3 (C2), 159.3 (C4"), 150.2 (C2'), 139.6 (C4', 9'), 123.9 (C6', 7'), 119.2 (C1"), 116.7 (C2", 3", 5", 6"), 115.3 (C5', 8'), 36.2 (CS); MS, *m/z*: 324.05 (100.0%, M^+), 325.06 (18.9%, M^++1), 326.07 (6.3%, M^++2), $(M)^+$ found = 324.05, $C_{16}H_{12}N_4O_2S$ requires 324.07.

(E)-2-((1H-benzo[d]imidazol-2-ylthio)methyl)-5-styryl-1,3,4-oxadiazole 5h:

Prepared from cinnamic acid (1.48 g, 0.01 mol) as yellowish powder; yield: 60%; IR, cm^{-1}: 3353.65 (NH str), 3085.45 (ArCH str), 1633.22 (C=N str), 1386.04 (NH bend), 1252.97 (COC str), 739.95 (CS str); ^1H NMR, δ 11.58 (s, 1H, 1'H), 7.87-7.85 (m, 4H, 5', 8', 2'', 6''H), 7.53-7.32 (m, 5H, 6', 7', 3'', 4'', 5''H), 7.28 (dd, *J*=14.35 Hz, 2H, olefinicH), 3.86 (s, 2H, SCH_2); ^{13}C NMR, δ 163.2 (C5), 158.6 (C2), 151.6 (C2'), 140.3 (C4', 9'), 137.8 (C1"), 134.1 (C olefinic), 129.2 (C2'', 3'', 5", 6"), 128.0 (C4"), 125.6 (C olefinic), 123.2 (C6',7'), 115.6 (C5',8'), 35.4 (CS); MS, *m/z*: 334.10 (100.0%, M^+), 335.09 (21.3%, M^++1), 336.07 (5.6%, M^++2), $(M)^+$ found = 334.10, $C_{18}H_{14}N_4OS$ requires 334.09.

2-(5-((1H-benzo[d]imidazol-2-ylthio) methyl)-1,3,4-oxadiazol-2-yl)phenol 5i:

Prepared from salicylic acid (1.38 g, 0.01 mol) as black powder; yield: 47%; IR, cm^{-1}: 3509.25 (OH str), 3200.26 (NH str), 3085.45 (ArCH str), 1603.07 (C=N str), 1345.49 (NH bend), 1244.69 (COC str), 740.10 (CS str); ^1H NMR, δ 10.52 (s, 1H, 1'H), 8.02 (s, 1H, OH), 7.69-7.62 (m, 3H, 5', 8', 6"H), 7.28-7.0 (m, 5H, 6', 7', 3'', 4'', 5''H), 3.51 (s, 2H, SCH_2); ^{13}C NMR, δ 166.1 (C5), 161.8 (C2), 158.2 (C2"), 150.6 (C2'), 139.8 (C4', 9'), 130.5 (C4"), 127.8 (C6"), 123.3 (C6', 7'), 122.2 (C5"), 118.6 (C3"), 115.7 (C5',8'), 109.0 (C1"), 33.8 (CS); MS, *m/z*: 324.05 (100.0%, M^+), 325.06 (20.2%, M^++1), 326.04 (3.0%, M^++2), $(M)^+$ found = 324.05, $C_{16}H_{12}N_4O_2S$ requires 324.07.

3-(5-((1H-benzo[d]imidazol-2-ylthio)methyl)-1,3,4-oxadiazol-2-yl)-1-cyclopropyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1H)-one 5j:

Prepared from ciprofloxacin (3.31 g, 0.01 mol) as yellowish powder; yield: 61%; IR, cm^{-1}: 3200.26 (NH str), 3085.45 (ArCH str), 2973 (AliCH str), 1616.68 (C=N str), 1363.81 (NH bend), 1260.65 (COC str), 1178.33 (CF str), 741.62 (CS str); ^1H NMR, δ 11.23 (s, 1H, 1'H), 8.67 (s, 1H, FCH), 7.85 (d, *J*=8.8 Hz, 2H, 5', 8'H), 7.52 (s, 1H, NCH, 1,4-dihydropyridine), 7.23 (d, *J*=8.8 Hz, 2H, 6', 7'H), 6.22 (s, 1H, FC-CH), 4.24-4.18 (m, 3H, cyclopropyl and SCH_2), 3.48 (t, 4H, $N(CH_2)_2$ piperazinyl), 2.81 (t, 4H, $N(CH_2)_2$ piperazinyl), 2.54 (s, 1H, piperazinyl), 1.38-1.18 (m, 4H, cyclopropyl); ^{13}C NMR, δ 176.3 (CO, 1,4-dihydropyridine), 167.4 (C5), 159.0 (C2), 154.2 (CF), 150.1 (C2'), 148.3 (C-N=Ar), 142.2 (=C-N 1,4-dihydropyridine), 139.6 (C4', 9'), 135.0 (=C-N condensed C of 1,4-dihydropyridine), 124.3 (C6', 7'), 118.1 (C condensed C of 1,4-dihydropyridine), 116.3 (=C 1,4-dihydropyridine), 113.2 (C5', 8'), 110.8 (FC=C), 103.8 (C Ar), 49.5 (N=C piperazinyl), 46.3 (C-NH piperazinyl), 36.1 (CS), 33.9 (NC cyclopropyl), 8.1 (C cyclopropyl); MS, *m/z*: 515.17 (100.0%, M^+), 516.16 (31.7%, M^++1), 517.18 (10.4%, M^++2), 518.18 (1.8%), $(M)^+$ found = 515.17, $C_{26}H_{24}FN_7O_2S$ requires 515.19.

Preparation of pyridazine and phthalazine derivatives 5k-m:

A mixture of compound 4 (2.67 g, 0.012 mol), succinic anhydride (1.0 g, 0.01 mol) for compound 5k, phthalic anhydride (1.48 g, 0.01 mol) for 5l and tetrachlorophthalic anhydride (2.85 g, 0.01 mol) for 5m in absolute ethanol (5 ml) and glacial acetic acid (0.28 ml, 0.005 mol) was refluxed for 3 h and cooled. The reaction mixture was poured into ice water mixture and neutralized with dilute $NaHCO_3$. The solid obtained was filtered and recrystallized from ethanol (95%).

1-(2-(1H-benzo[d]imidazol-2-ylthio)acetyl) pyridazine-3,6-dione 5k:

Pink powder; yield: 27%; IR, cm^{-1}**:** 3349.19 (NH str), 3085.45 (ArCH str), 3072.56 (AliCH str), 2986 (AliCH str), 1831.94 (C=O str), 1638.19 (C=N str), 1329.65 (NH bend), 731.71 (CS str); ^1H NMR, δ 12.54 (s,1H, 1'H), 10.41 (s, 1H, 2H), 8.02 (d, *J*=8.4 Hz, 2H, 5',8'H), 7.62 (d, *J*=8.4 Hz, 2H, 6', 7'H), 4.36 (s, 2H, SCH2), 2.55 (s, 4H, 4, 5H); ^{13}C NMR, δ 175.2 (C3), 173.4 (C6), 168.8 (C=O), 148.1 (C2'), 139.5 (C4', 9'), 124.0 (C6', 7'), 115.3 (C5', 8'), 38.6 (CS), 29.1 (C4), 26.2 (C5); MS, *m/z*: 304.03 (100.0%, M^+), 305.04 (15.9%, M^++1), 306.05 (6.2%, M^++2), $(M)^+$ found = 304.03, $C_{13}H_{12}N_4O_3S$ requires 304.06.

2-(2-(1H-benzo[d]imidazol-2-ylthio)acetyl)-2,3-dihydrophthalazine-1,4-dione 5l:

Black powder; yield: 35%; IR, cm^{-1}: 3383.12 (NH str),

3166.96 (ArCH str), 1687.55 (C=O str), 1655.62 (C=N str), 1370.07 (NH bend), 739.90 (CS str); ^{1}H NMR, δ 11.62 (s, 1H, 1'H), 7.86 (s, 1H, 2H), 7.60-7.56 (m, 4H, 5, 6, 7, 8H), 7.51 (d, *J*=8.6 Hz, 2H, 5', 8'H), 7.33 (d, *J*=8.6 Hz, 2H, 6', 7'H), 4.28 (s, 2H, SCH_2); ^{13}C NMR, δ 169.3 (C=O), 168.1 (C3), 159.2 (C10), 147.8 (C2'), 140.1 (C4', 9'), 132.5 (C6, 7), 129.2 (C4, 9), 124.2 (C5, 8), 123.5 (C6', 7'), 115.9 (C5', 8'), 38.9 (CS); MS, *m/z*: 352.11 (100.0%, M^+), 353.10 (21.3%, M^++1), 354.08 (6.3%, M^++2), $(M)^+$ found = 352.11, $C_{17}H_{12}N_4O_3S$ requires 352.06.

2-(2-(1H-benzo[d]imidazol-2-ylthio)acetyl)-5,6,7,8-tetrachloro-2,3-dihydrophthalazine-1,4-dione 5m:

Yellowish white powder; yield: 39%; IR, cm^{-1}: 3410.36 (NH str), 3053.17 (ArCH str), 1700.26 (C=O str), 1329.92 (NH bend), 739.85 (CS str), 648.26 (CCl str); ^{1}H NMR, δ 11.58 (s, 1H, 1'-H), 8.63 (s, 1H, 2-H), 7.64 (d, *J*=8.62Hz, 2H, 5', 8'-H), 7.36 (d, *J*=8.62 Hz, 2H, 6', 7'-H), 4.17 (s, 2H, SCH_2); ^{13}C NMR, δ 168.7 (C=O), 167.9 (C3), 159.3 (C10), 147.8 (C2'), 139.4 (C4', 9'), 135.8 (C6, 7), 133.6 (C4, 9), 128.9 (C5, 8), 124.0 (C6', 7'), 115.7 (C5', 8'), 38.3 (CS); MS, *m/z*: 487.93 (75.8%, M^+), 488.91 (13.9%, M^++1), 489.9 (100%, M^++2), 490.9 (20.6%), 491.8 (52.8%), 492.9 (10.0%), 493.8 (12.7%), 494.9 (2.7%), $(M)^+$ found = 487.93, $C_{17}H_8Cl_4N_4O_3S$ requires 487.91.

Antimicrobial activity:

In the initial tube containing 380 µl of BHI broth, 20 µl of compound (representing 100 µg of compound) from the stock solution prepared by dissolving 10 mg of test compound in 2 ml DMSO, was added. For dilutions, 200 µl of BHI broth was added into the next 9 tubes separately. Then from the initial tube, 200 µl was transferred to the second tube containing 200 µl of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube (second tube) 200 µl was transferred to third tube to make 10^{-2} dilutions. The serial dilution was repeated up to 10^{-9} dilutions for each compound. In each serially diluted tube, 200 µl of microbial culture suspension in BHI (10^5 bacteria/fungi per ml) was added. Ciprofloxacin/fluconazole and control tests with solvent DMSO were performed under similar condition for comparison. The tubes were incubated at 37° for antibacterial and 25° for antifungal activities for 24 h and observed for turbidity. The MIC was defined as lowest concentration of compound, which prevented the formation of turbidity.

Antitubercular activity:

First, 200 µl of sterile deionised water was added to all outer perimeter wells of sterile 96 wells plate to minimize the evaporation of medium in the test wells during incubation. The wells received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds dissolved in DMSO were made directly on plate from 100 to 0.2 µg/ml. The above said wells were inoculated with 100 µl of 2000 cfu/ml of organisms in Middlebrook 7H9 broth. Plates were covered and sealed with Parafilm and incubated at 37° for 5 d. After this, 25 µl of freshly prepared 1:1 mixture of AlamarBlue reagent and 10% Tween 80 was added to the plate and incubated for 24 h. The same method was followed for control DMSO and INH. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration, which prevented the colour change from blue to pink.

RESULTS AND DISCUSSION

The synthetic routes leading to title compounds 5a-j and 5k-m were depicted in figs. 1 and 2. Well versed with the intrinsic antimicrobial property of sulphur, 1H-2-mercapto benzimidazole 1 was aptly used as the starting material to incorporate 1H-benzimidazol-2-ylthio methyl motif into the target molecules. 2-(1-H-benzo[d] imidazol-2ylthio) acetic acid 2 was obtained through William's reaction of 1 with chloroacetic acid in the presence of sodium hydroxide[8,25]. On treatment with thionyl chloride, 2 yielded the acid chloride, 2-(1-H-benzo[d]imidazol-2ylthio)acetyl chloride 3[26,27]. 2-(1-H-benzo[d]imidazol-2-ylthio)aceto hydrazide 4 was prepared by reacting 3 with hydrazine hydrate[28]. The 4 was further cyclodehydrated with different substituted aromatic acids in presence of phosphorous oxychloride as dehydrating agent to generate 2-(benzimidazol-2ylthiomethyl)-5-aryl-1,3,4-oxadizoles 5a-j in moderate to good yields. The other dehydrating agents used for this purpose include sulphuric acid, phosphoric acid, trifluoroacetic acid, phosphorus pentachloride, phosphorus pentoxide, thionyl chloride and milder reagents such as carbodiimide derivatives, TsCl/pyridine, trimethylsilyl chloride, Ph_3O/Tf_2O, PPh_3/CX_4 (X=Cl, Br, I) and Burgess reagent[29,30]. The mechanism of formation of compounds 5a-j[30] is outlined in fig. 3. In another route, 4 on condensation with succinic, phthalic and tetrachlorophthalic anhydrides in presence of catalytic amount of acetic acid resulted in the formation of corresponding pyridazine or phthalazine diones[31]

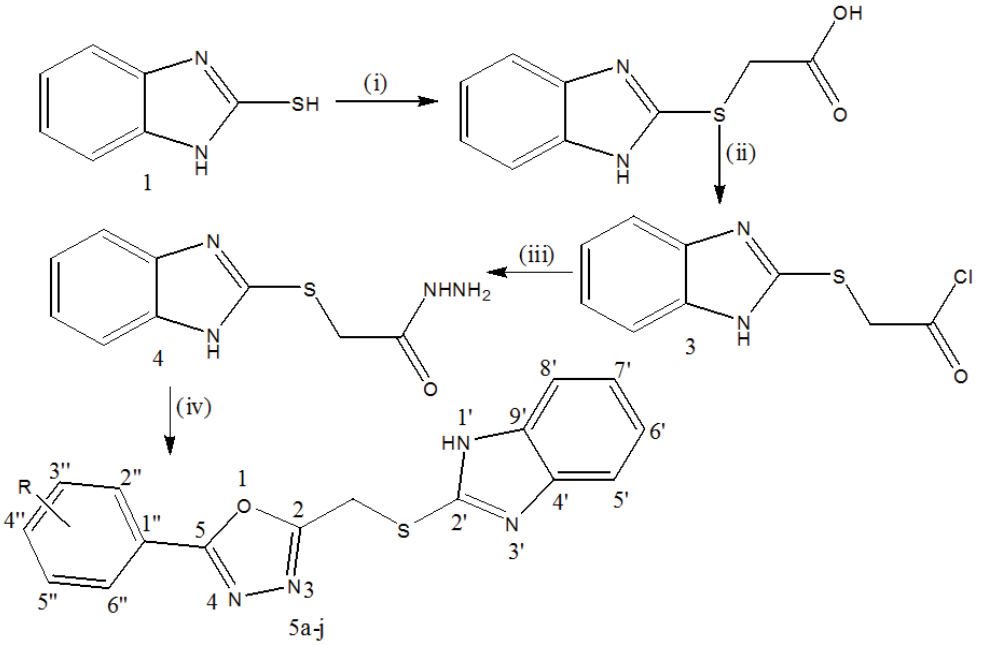


Fig. 1: Synthetic route for compounds 5a-j
Reagents: i. $ClCH_2COOH/NaOH$; ii. $SOCl_2$; iii. $NH_2NH_2H_2O$; iv. $ArCOOH/POCl_3$

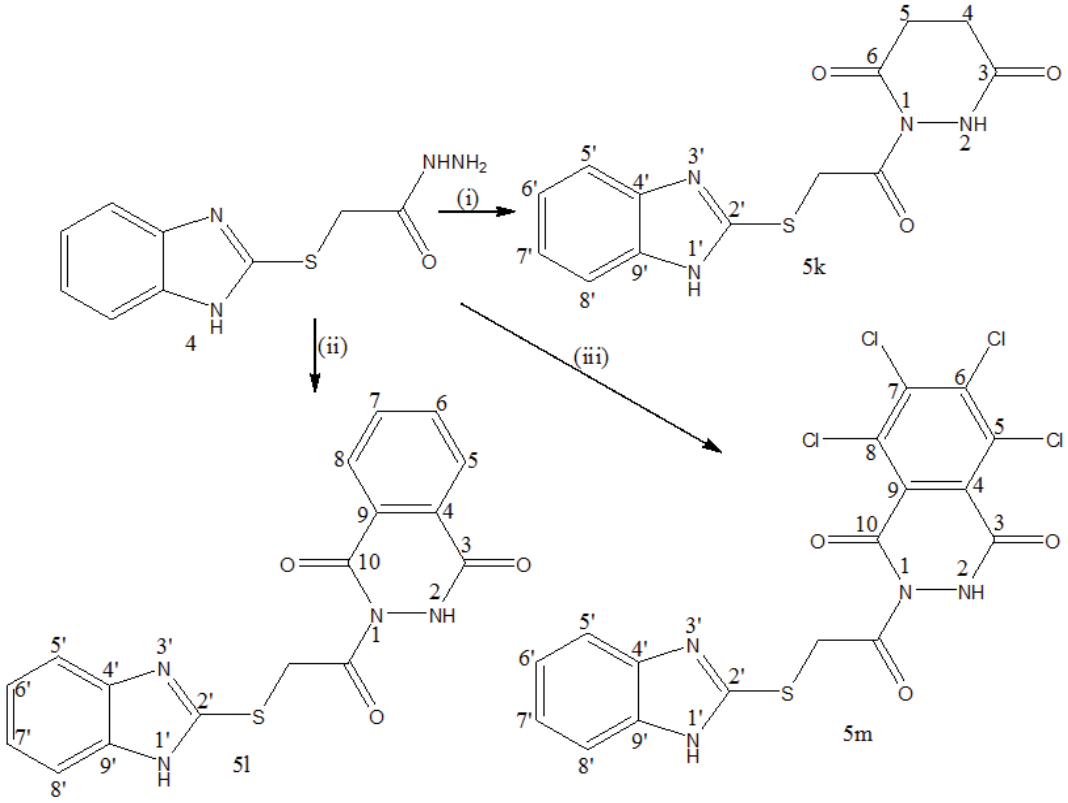


Fig. 2: Synthetic route for compounds 5k-m
Reagents: i. succinic anhydride/AcOH; ii. phthalic anhydride/AcOH; iii. tetrachl orophthalic anhydride/AcOH

viz.,1-(2-(1H-benzo[d]imidazol-2-ylthio)acetyl) pyridazine/(tetrachloro)phthalazine diones 5k, 5l and 5m, respectively in moderate yields. The synthesized compounds were purified by recrystallization from ethanol as more or less amorphous powders. The R_f values and melting points of the compounds 5a-m are presented in Table 1.

The structure of the synthesized compounds was determined on the basis of their IR, NMR and mass spectral data. The IR spectrum of 3 exhibited the

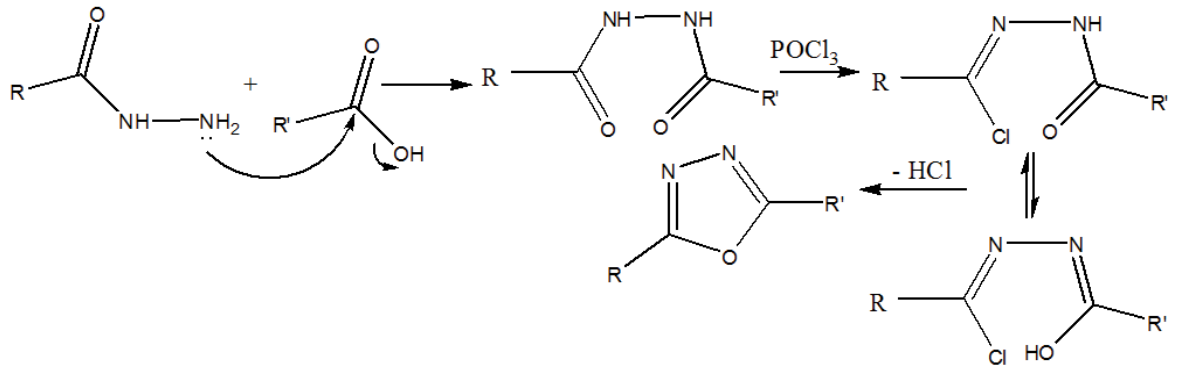
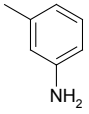
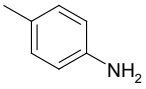
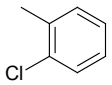
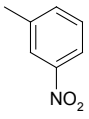
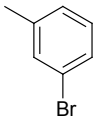
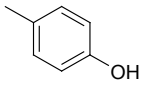
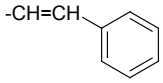
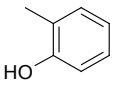
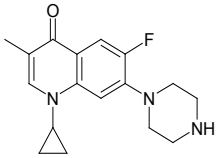


Fig. 3: Mechanism of formation of 2,5-disubstituted-1,3,5-oxadiazoles

TABLE 1: PHYSICOCHEMICAL DATA OF COMPOUNDS (5 a-m)

Entry	Ar	R_f value	MP, °
5a		0.70 (Pet. ether:benzene 7:3)	190-192
5b		0.56 (Pet. ether:benzene 8:2)	272-274
5c		0.53 (Pet. ether:benzene 7:3)	226-228
5d		0.72 (Pet. ether:benzene 2:8)	282-284
5e		0.91 (Pet. ether:benzene 3:7)	167-169
5f		0.55 (Pet. ether:benzene 1:9)	171-173
5g		0.36 (Pet. ether:benzene 3:7)	211-213
5h		0.62 (Pet. ether:benzene 8:2)	232-234
5i		0.35 (Pet. ether:benzene 3:7)	278-279
5j		0.45 (Pet. ether:benzene 3:7)	213-214
5k	--	0.63 (Pet. ether:benzene 3:7)	295-296
5l	--	0.65 (Pet. ether:benzene 3:7)	234-236
5m	--	0.52 (Pet. ether:benzene 3:7)	284-285

bands at 1792 cm^{-1} due to C=O stretch, 682 cm^{-1} ascribing to C-Cl stretch, 769 cm^{-1} corresponding to C-S stretch, 3145 cm^{-1} pertaining to aromatic C-H stretch and 3452 cm^{-1} attributing to N-H stretch. The compounds 5a-j showed bands in IR spectra at 3136-3383 cm^{-1} characteristic to NH stretch, 3053-3166 cm^{-1} because of aromatic C-H stretch, 1598-1655 cm^{-1} due to C=N stretch, 1252-1265 cm^{-1} corresponding to C-O-C asymmetric stretch and 731-744 cm^{-1} due to C-S stretch. In their IR spectra, 5k-m displayed bands at 3349-3410 cm^{-1} pertaining to N-H stretch, 1657-1687 cm^{-1} ascribing to C=O stretch, 1638-1655 cm^{-1} corresponding to C=N stretch, 1329-1370 cm^{-1} attributing to N-H deformation and 731-739 cm^{-1} due to C-S stretch. The ^{1}H NMR spectra of 5a-m exhibited a broad singlet at δ 12.71-10.41 representing benzimidazole NH, aromatic hydrogens resonated in the range of δ 8.5-6.10, a singlet at δ 4.6-3.9 corresponding to two hydrogens of SCH_2 and at δ ~8.0 representing pyridazine/phthalazine NH amongst other protons. The ^{13}C NMR spectra of compounds exhibited peaks due to aromatic carbons at δ ~160-110, carbonyl carbons at δ 176-165, carbon of SCH_2 at δ ~40 and aliphatic carbon atoms at δ ~50-8. The M^+ and/or M^++1 and M^++2 peaks showed by 5a-m in mass spectra were in close agreement with their molecular weights.

The title compounds 5a-m were evaluated for antimicrobial activity against *S. aureus*, *E. faecalis* (Gram-positive bacteria), *K. pneumonia*, *E. coli* (Gram-negative bacteria) and *C. albicans* and *A. niger* (fungi) by serial dilution method[32] on BHI broth medium. The antimicrobial screening results presented in Table 2 were mean of triplicate reading. Only 5a showed MIC 6.25 μg/ml, indicating better activity than standard drug ciprofloxacin whereas, 5j with 12.5 μg/ml, 5e and 5f with 25 μg/ml displayed moderate activity against *S. aureus*. Electron withdrawing substituents such as nitro and bromo sustained the activity. All the compounds showed potent activity against *E. faecalis*, exhibiting 0.2 μg/ml MIC in comparison to 10 μg/ml for ciprofloxacin. It seems, substitutions on phenyl ring at the 2-position of 1,3,4-oxadiazole has no effect on activity against *E. faecalis*. All compounds tested had very poor activity against *E. coli* exhibiting no dependency on substituents except one compound. Only 5j showed 0.2 μg/ml MIC against *K. pneumoniae*, indicating a greater potency than ciprofloxacin (MIC 10 μg/ml). Since 5j contains ciprofloxacin fragment in it, the activity seems to be better than ciprofloxacin itself. Eventually, 5j emerged out to possess good activity against both Gram-positive and Gram-negative bacteria. In general, the title compounds displayed good activity against Gram-positive organisms relative to against the Gram negative. This could perhaps be due to poor cell wall penetration of the compounds.

Against *C. albicans*, 5g, 5l and 5m exhibited good antifungal activity (MIC 50 μg/ml) than rest of the compounds. However, they were less active than standard antifungal fluconazole (MIC 30 μg/ml). Phthalazine derivatives were found to be better acting than their pyridazine counterpart. All the compounds displayed an outstanding antifungal activity with MIC

TABLE 2: ANTIMICROBIAL ACTIVITY OF COMPOUNDS (5 a-m)

Compound	CLogP	MIC, μg/ml						
		S. aureus	*E. faecalis*	*E. coli*	*K. pneumoniae*	*A. niger*	*C. albicans*	*M. tuberculosis*
5a	2.82	6.25	0.2	100	100	0.2	100	12.8
5b	1.86	50	0.2	100	100	0.2	100	25.6
5c	1.50	50	0.2	100	100	0.2	100	51.2
5d	3.31	50	0.2	100	100	0.2	100	51.2
5e	2.63	25	0.2	100	100	0.2	100	51.2
5f	3.71	25	0.2	100	100	0.2	100	51.2
5g	2.44	50	0.2	100	100	0.2	50	>100
5h	3.41	50	0.2	100	100	0.2	100	51.2
5i	2.14	100	0.2	100	100	0.2	100	12.8
5j	1.14	12.5	0.2	100	0.2	0.2	100	25.6
5k	0.79	100	0.2	100	100	0.2	100	51.2
5l	3.88	100	0.2	100	100	0.2	50	51.2
5m	6.37	100	0.2	100	100	0.2	50	51.2
Ciprofloxacin	-0.72	10	10	10	10	---	---	---
Fluconazole	0.52	---	---	---	---	30	30	---
INH	-0.67	---	---	---	---	---	---	0.4

0.2 µg/ml against *A. niger* than fluconazole. Here also, the activity is found to be independent of substituents. Among the title compounds, 5j showed selectivity towards bacteria whereas; phthalazine 5l and 5m displayed somewhat selectivity towards fungi.

The *in vitro* antitubercular activity of compounds was assessed against *M. tuberculosis H37Rv* by microplate alamar blue assay[33]. The activity data is shown in Table 2. The compounds showed very weak antitubercular activity. Only 5a and 5i showed MIC 12.8 µg/ml vis-à-vis 0.4 µg/ml for standard drug INH. The poor antitubercular activity by title compounds could probably be due to their lower lipophilicity, indicated by their C log P values, and thereby reduced cell wall permeation. Thus, among the title compounds, 5a with phenyl substitution and 5i with 2-hydroxy phenyl substitution emerged to be moderately acting against all the tested bacteria, fungi and mycobacterium.

In conclusion, a new series of 1H-benzimidazol-2-ylthiomethyl moiety incorporated hybrid molecules containing 2-substitutituted 1,3,4-oxadiazole, pyridazin-3,6-dione and phthalazin-1,4-dione were synthesized successfully by a facile protocol via acid hydrazide intermediate and were spectrally characterized. The target compounds showed excellent to moderate to poor preliminary *in vitro* antimicrobial and very feeble *in vitro* antitubercular activity against tested bacterial and fungal strains. The results showcased the significance of molecular hybridisation leading to bioactive hybrid molecules. However, synthesis of many more derivatives in the series and their activity testing would usher new trends in structural optimization of these compounds. Further, quantitative structure-activity relationship (QSAR) studies would probably give a better insight into biochemical mode of action and pattern of activity of the synthesized compounds. Also, the template considered in the study is found to have anticancer effect[34], the title compounds would also be explored as anticancer agents in addition to antimicrobial molecules warranting a powerful incentive for further research.

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Conflict of interest:

The authors declare no conflict of interest with respect to this work.

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Nil.

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